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Genetic Effects from Exposure to Hazardous Agents

by Jack Favor

Mammalian germ cell stages exhibit differences in DNA synthesis activity, capability to repair DNA damage, and chromosome-associated proteins. The sensitivity to mutation induction may be influenced by such factors as the accessibility of DNA to chemical mutagens, the interval between DNA damage induction and the next round of DNA replication, and the repair of DNA damage. Such qualitative and quantitative differences indicate the complexities of mutation induction *in vivo* and emphasize that no single *in vitro* test system can adequately represent the *in vivo* situation. Therefore, germ-cell mutagenesis in humans can most adequately be represented by an *in vivo* mammalian germ-cell test system. Information regarding the mechanisms of mutation induction in germ cells of the mouse, appropriate mutation test systems available in the mouse, as well as principles of chemical mutagenesis in the mouse and their implications for an adequate human genetic risk estimation will be discussed.

Introduction

The mechanism of mutation induction is a complex process that may involve metabolic activation or detoxification of a chemical mutagen, the induction of DNA adducts, and DNA repair and mutation fixation. Further, gametogenesis in mammals is associated with cell differentiation at the genetic, morphologic, and metabolic levels. Germ cell stages exhibit differences in DNA synthesis activity, capability to repair DNA damage, and chromosome-associated proteins. The sensitivity to mutation induction may be influenced by such factors as the accessibility of DNA to chemical mutagens, the interval between DNA damage induction and the next round of DNA replication, and the repair of DNA damage. Such qualitative and quantitative differences indicate the complexities of mutation induction *in vivo* and emphasize that no single *in vitro* test system can adequately represent the *in vivo* situation. Therefore, germ-cell mutagenesis in humans can most adequately be represented by an *in vivo* mammalian germ-cell test system. Information regarding the mechanisms of mutation induction in germ cells of the mouse, appropriate mutation test systems available in the mouse, and principles of chemical mutagenesis in the mouse and their implications for adequate human genetic risk estimation are discussed. The discussions rely on experimental information for gene mutations and do not include data for chromosome structural alterations.

GSF-Institut für Säugetiergenetik, D-8042 Neuherberg, Germany.

Molecular Characterization of Ethylnitrosourea-Induced Mutations

To date, six independent ethylnitrosourea (ENU)-induced mutations in germ cells of the mouse have been characterized by direct DNA sequencing or deduced from their amino acid substitutions (*1–4*; S. E. Lewis, personal communication; J. Peters, personal communication), and all have been shown to be base substitutions affecting an AT site. The O^6-ethylguanine adduct has been suggested to be the most relevant DNA adduct that leads to mutation (*5,6*). The mutation resulting from O^6-ethylguanine mispairing is predicted to be a GC to AT base substitution. This predicted mechanism of ENU mutagenesis has been experimentally confirmed in *E. coli* (*7*) and Salmonella (*8*), and also predominates in Drosophila (*9–11*), as well as mammalian cells in culture (*12,13*). Thus, the most relevant DNA adduct in germ cells of the mouse differs from that in other test systems. A wide variety of base adducts may be formed after the interaction of ENU with DNA (*14,15*). Although the O^6-ethylguanine adduct is the most frequent base ethylation that leads to mispairing, its lack of involvement in ENU germ-cell mutagenesis suggests an efficient repair mechanism. In contrast, there is a lack of evidence for an efficient repair mechanism of O-ethyl pyrimidine adducts, which would also lead to base mispairing (*16*). Results therefore emphasize the complexities of germ-cell mutagenesis in mammals and support the assumption that the best experimental system to represent germ-cell mutagenesis in humans is *in vivo* germ-cell test systems in laboratory animals.

Mammalian Germ Cell Mutagenesis

Table 1 lists a number of mutation test systems developed to screen for transmitted, genetically validated germ-cell mutations in the mouse. A variety of mutational classes may be recovered by different methods, including recessive and dominant mutations and biochemical or immunological mutants. The methods are not mutually exclusive, so experiments may be designed to systematically screen for more than one genetic end point in the same experimental population (*17*).

The specific-locus method developed by Russell (*18*) is the most efficient method to screen for transmitted germ-cell mutations in the mouse. It has provided virtually all experimental data on factors affecting the mutation process in germ cells of mammals. The advantage of the specific-locus test is that the methods to screen for mutations are simple and fast, so the large numbers of animals required to study an infrequent event such as mutation can be examined. Finally, the animals are alive at examination and mutations can be subjected to a genetic confirmation test. The specific-locus method in the mouse has been discussed in detail (*19–21*). For radiation, dose, dose rate, dose fractionation, dose fractionation interval, radiation quality, and germ-cell stage have all been shown to affect the induced specific-locus mutation rate. For chemical mutagens, germ-cell stage specificity plays an important role. See the extensive review by Lyon (*22*). In contrast to radiation where differences in the relative sensitivity to mutation induction exist among the different spermatogenic stages, for chemicals, qualitative and quantitative differences in the sensitivity to mutation induction among the stages may exist. Table 2 illustrates this principle for a group of ethylating agents studied in Neuherberg using the specific locus test. The mutagens diethyl sulfate (DES) and ethyl methanesulfonate (EMS) are effective in the late spermatid and early spermatozoa stages and are not effective in spermatogonia. Methyl methanesulfonate (MMS) has a pattern of germ-cell stage sensitivity to mutation induction in the specific-locus test identical to DES and EMS (*23*). ENU, by contrast, is mutagenically active in post-spermatogonial stages, but mutations mainly occur as mosaics. ENU is most effective in stem-cell spermatogonia.

Table 1. Tests developed to detect transmitted mutations in germ cells of the mouse.

Test	Methods	Reference
Specific locus	F_1, external visible traits	(*18*)
Specific locus	F_1, external visible traits	(*40*)
Recessive lethal	F_2 backcross, embryonic	(*41*)
Dominant visibles	F_1, external visible traits	(*20*)
Dominant skeletal	F_1, skeletal preparations	(*42,43*)
Dominant cataract	F_1, ophthalmological examination	(*44*)
Dominant fitness	F_2, litter size effects	(*45*)
Electrophoretic	F_1, variant protein electrophoretic pattern	(*46–50*)
Enzyme activity	F_1, specific enzyme activity	(*51,52*)
Histocompatibility	F_1, skin graft rejection by day 80	(*53*)

Table 2. Germ-cell-stage sensitivity to mutation induction in male mice.

Interval[a]	EMS (*25*)	DES (*54*)	ENU (*55,56*)
1–4	0/10,950	0/ 5,617	
5–8	7/ 8,276	6/ 5,271	5/7,611
9–12	8/ 7,624	2/ 6,048	
13–16	2/10,816	0/ 6,375	3/ 4,763
17–20	1/11,586	1/ 6,694	
21–42	0/ 5,138	0/ 2,609	2/ 3,168
>43	2/28,181	0/13,551	173/58,211

Abbreviations: EMS, ethyl methanesulfonate; DES, diethyl sulfate; ENU, ethylnitrosurea.

[a]Interval during which treated males were mated to untreated females; time interval expressed as days after treatment.

Table 3. Mutagenic activity of chemicals with conclusive specific locus mutation test results in stem cell spermatogonia and post-spermatogonial stages of the mouse.[a]

Chemical	Post-spermatogonia	Spermatogonia
Ethylnitrosourea	+	+
Methylnitrosourea	+	+
Procarbazine	+	+
Triethylenemelamine	+	+
Acrylamide	+	−
Chlorambucil	+	−
Cyclophosphamide	+	−
Diethyl sulfate	+	−
Ethyl methanesulfonate	+	−
Mitomycin C	−	+
6-Mercaptopurine	−	−
Adriamycin	−	−
Platinol	−	−
Urethane	−	−

[a]Adapted from Ehling (*57,58*), Lyon, (*22*), and Russell (*59*).

Table 3 lists the qualitative results for all chemicals with conclusive specific-locus mutation results in both post-spermatogonial stages and stem-cell spermatogonia. All possible patterns of stage sensitivity are apparent. One group of chemicals is active in both post-spermatogonial stages and stem-cell spermatogonia. A second group of chemicals is active in post-spermatogonial stages but not in stem-cell spermatogonia. Mitomycin C is not active in post-spermatogonia but is active in stem-cell spermatogonia. Finally, a fourth group of chemicals is not active in post-spermatogonial stages or in stem cell spermatogonia. For a complete assessment of the mutagenic activity of a chemical in germ cells of mammals, all germ-cell stages must be adequately tested (*24–27*). Further, a risk assessment of a chemical mutagen must take into account the germ-cell stage pattern of mutagenic activity as well as the mode of exposure, chronic or acute.

Dose Response

Knowledge of the dose response of a chemical mutagen is required for an adequate estimation of mutagenic effect due to exposure. Otherwise, an assumption of the dose response, usually linearity, must be invoked for a risk estimation.

Two chemical mutagens have been extensively studied in stem-cell spermatogonia of the mouse with the specific-

locus test: procarbazine and ENU. Results for procarbazine indicate a humped dose response in which there is a dose-related increase in mutagenic response that reaches a maximum and beyond which the mutagenic effect decreases (*28*). For ENU, a threshold or quasi-thresholded dose response has been demonstrated (*29*). That both data sets indicate nonlinear dose responses emphasizes the difficulties in estimating genetic risk due to exposure. These difficulties are especially pronounced if there are large differences between the experimental dose ranges on which extrapolation is based and the actual exposure dose on which risk is estimated. Depending on the shape of the dose response and the experimental dose interval relative to the dose for which risk is estimated, overestimation or underestimation of the true risk is possible if the extrapolation is based on the assumption of linearity.

Relevant Genetic End Points

In a randomly mating, natural population, newly induced recessive mutations will result in a mutant phenotype only when they occur as a homozygote. This is a function of the square of the allelic frequency, and in the first generation after radiation exposure would result in an expected negligible increase in the frequency of mutant individuals. Dominant mutations, by definition, express phenotypic effects as a heterozygote. Thus, newly induced dominant mutations would be detected in the F_1 population after radiation exposure regardless of the genotype or mating scheme in the parental generation. Table 4 lists the estimated incidence and spontaneous mutation frequency for various classes of genetic disorders in man. The frequency of spontaneous mutations is estimated based on the assumption of mutation-selection equilibrium. Thus, it is directly proportional to the selection coefficient of a mutant allele in the population and the observed incidence of mutants. It is evident from Table 4 that in human populations, dominant disorders have a significant incidence. A large proportion of dominant disorders are due to newly occuring spontaneous mutations as compared to recessive or irregularly inherited disorders and are a major concern in estimating the genetic risk due to an increased mutation rate. Kacser and Burns (*30*) have argued that a mutation leading to a simple loss or increase of the normal gene function should be recessive, whereas a mutation leading to an alteration of the gene product that interferes with the normal gene product would be dominantly expressed. A more limited spectrum of DNA alterations may result in a dominant allele rather than the broader spectrum of DNA lesions resulting in loss of gene function, which are expressed recessively. Thus, it can be hypothesized that the mutational events leading to a recessive or a dominant allele may be qualitatively different.

Table 4. Genetic disorders in man (*60*).

Class	Incidence per 10^6	Spontaneous mutation rate per 10^6
Dominant and X-linked	10,000	1,500
Recessive	2,500	–
Irregularly inherited	90,000	450

Therefore, it would be precarious to base all characterizations of the mutational process in germ cells of mammals on results with recessive alleles given the fact that dominant alleles pose the major genetic risk and that the mutational event leading to recessive or dominant alleles may differ (*31*). For example, we have recently shown the radiation doubling dose for induced dominant cataract mutations to be significantly higher than the doubling dose for induced recessive specific-locus mutations (*32*; Löbke et al., in preparation).

Genetic Risk Estimation

Two extrapolation procedures have been developed to estimate the genetic risk in man based on experimental data from the mouse. The first, called the doubling dose approach, is based on the estimate in the mouse specific-locus test of the dose that results in an induced mutation rate equal to the per-generation spontaneous mutation rate and on an estimate of the spontaneous mutation rate in humans. An indirect method to estimate the spontaneous mutation rate to dominant alleles in humans has been outlined by Childs (*33*). Given the population incidence and selection coefficient of a dominant disorder, the mutation rate is calculated based on the assumption of mutation-selection equilibrium. For congenital cataract, the population incidence is 4×10^{-5}, the selection coefficient is 0.3, and the spontaneous mutation rate is estimated to be 0.6×10^{-5}. For the entire class of dominant deleterious mutations, approximately 14% of affected individuals are estimated to be due to a newly occurring spontaneous mutation. The number of induced dominant genetic disorders due to mutagenic exposure in humans is calculated as follows:

$$\text{Induced cases} = (\text{Exposure dose/Doubling dose}) \times \text{Spontaneous mutation rate for dominant deleterious mutations.}$$

The second extrapolation procedure to estimate genetic risk in man is called the direct approach. It is based on an estimate of the induced mutation rate to dominant alleles in mice and on an estimate of the total number of loci in humans that result in dominant genetic disorders relative to the number of loci in humans controlling the indicator phenotype used in the mouse experimental studies to estimate the induced mutation rate (to date, skeletal and cataract mutations). The number of induced dominant genetic disorders due to mutagenic exposure in humans is calculated according to Ehling (*34,35*) as follows:

$$\text{Induced cases} = \frac{\text{Induced mutation rate per gamete per dose}}{} \times \frac{\text{Total dominant loci}}{\text{Indicator dominant loci}} \times \text{Dose}$$

The direct approach avoids the problem of basing the estimate of induced mutations for one genetic end point on experimental mutagenesis data on a different genetic end point. However, the estimate of the total number of loci resulting in dominant genetic disorders and the total number of cataract or skeletal loci in humans is critical. It

is based on the tabulations of dominant genetic disorders in humans (*36*). The value for total dominant genetic disorders is 1172 and for cataracts is 28. It must be considered that this categorization is based on distinct phenotypes with no genetic determinations that all distinct phenotypes are a result of distinct loci. The converse is also possible, i.e., similar phenotypes may result from distinct loci. Therefore, there is as yet no way to know if these values are under- or over estimates.

It should be recalled that the sensitivity to mutation induction by chemical mutagens in the mouse is germ-cell-stage specific. Thus, the doubling dose and induced mutation rate for dominant alleles determined for a chemical mutagen and used for an estimation of the genetic risk in man is germ-cell-stage specific. For an acute exposure, chemicals with a mutagenic effect confined to post-spermatogonial stages will have a transitory genetic risk confined to conceptions resulting from gametes that were exposed during the sensitive stages. Conceptions occurring from gametes that were exposed in the nonsensitive stages will have a genetic risk of zero associated with the particular exposure. For chemicals with a mutagenic effect in stem-cell spermatogonia, a permanent genetic risk will remain after an acute exposure because the stem-cell spermatogonia population constantly cycles to reestablish itself. For a chronic exposure, the genetic risk for a chemical will be the combined genetic risk of the chemical for all stages of spermatogenesis. For chemicals with mutagenic effects confined to post-spermatogonial stages, the genetic risk associated with chronic exposure will be equal to the genetic risk in the sensitive post-spermatogonial stages regardless of the duration of exposure. Further, upon cessation of exposure, the genetic risk will return to zero. For chemicals with mutagenic effects in stem-cell spermatogonia, the genetic risk associated with chronic exposure will constantly increase over the duration of the exposure and will remain at the final level after exposure ceases.

The complete assessment of a compound for mammalian germ-cell mutagenesis and the associated genetic risk would require results for treatment in female germ-cell stages. In addition to radiation, results for the sensitivity to induction of specific-locus mutations are only available for four chemicals: procarbazine (*37*), mitomycin C (*38*), triethylenemelamine (*39*), and ENU (*37*). As for male germ cells, results for female germ cells indicate a germ-cell-stage effect on the sensitivity to mutation induction, which may vary depending on the mutagenic treatment used. The dynamics of gametogenesis in females [see Searle (*20*) for review] include a relatively short period of mitotic proliferation in the early embryonic stage. Meiosis is initiated during embryogenesis and proceeds to the late diplotene stage shortly after birth. Oocytes remain in this stage until shortly before ovulation. This is the meiotic stage of primary importance for mutagenic hazard. Within this stage differences exist in sensitivity to cell killing and mutation induction depending on the stage of development of the associated follicle cells. In comparison to the stem-cell spermatogonia of males, the important stage in female gametogenesis may be characterized by the absence of cell division and DNA replication. As in stem-cell spermatogonia, oocytes are DNA repair competent. Thus, DNA damage is not likely to accumulate in oocytes and there may be a long interval between the induction of DNA damage and the next round of DNA replication, which occurs after fertilization.

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